Remarks

Applicant submits this Response in reply to the Final Office Action mailed December 1, 2005. Claims 1-2, 4-21 remain in the application. Claims 1, 2, 10, 14, 15 and 21 are presently amended to overcome the §112 and §103(a) rejections below. Claim 3 has been canceled and incorporated into Claim 1. No new matter has been added. For the reasons set forth below, Applicant respectfully submits that Claims 1-2, 4-21 are now in condition for allowance.

35 U.S.C. §112 Rejection

In the Office Action, the Examiner rejected Claims 1-21 under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Claim 1 - Part A is recited below with the language at issue underlined.

> A. a photonic band gap structure including an internal surface that defines a core region; wherein said internal surface of said photonic band gap structure is coated with a film formed of aplurality of molecules;"

With regard to Claim 1 - Part A, the Examiner states that the original specification fails to provide guidance as to any specific substances which have the ability to generate a signal in response to interacting with a "microorganism" that has been excited to interact with the film of molecules. The Examiner also states that the specification does recite that the molecules can be "conjugated polymer molecules" but again fails to set forth specific molecules that would be capable of functioning as recited in the claim and/or that would be know to one of ordinary skill in the art at the time of the filing of the instant invention.

The Applicant respectfully traverses the rejection of Claim 1 - Part A for the reasons stated below. As stated by the Examiner, a film formed of a plurality of molecules was described in the specification of the present Application as a "conjugated polymer molecule" (U.S. Published Application No. US20020155592 A1, p.1, para. 14; p. 2, para. 17; p.4, para. 41) The specification of the present Application expands upon the term "conjugated polymer molecule."

The film, in a preferred embodiment, can also be described as a "conjugated polymer biosensing film formed of conjugated polymer biosensor hybrid molecules" (U.S. Published Application No. US20020155592 A1, p.3, para. 38; p. 4, para. 49) The term "biosensing" or "biosensor" provides guidance as to the type of conjugated polymer molecule that would be used by a person of ordinary skill in the art.

The term "conjugated polymer molecule" and its use was well known in the prior art at the time of the filing of the present Application. Prior to the filing date of the present Application, the prior art explained how the usage of conjugated polymers provided provided the basis for a new class of highly sensitive biological and chemical sensors. (see Liaohai Chen, et al., *Highly Sensitive Biological and Chemical Sensors Based on Reversible Fluorescence Quenching in a Conjugated Polymer*, PNAS 96: 12287-12292 (October 26, 1999) ("Chen Article") A copy of the Chen Article is hereby attached as Exhibit A. In the Chen Article at p. 12287, PPV (Poly phenylene vinylene) is mentioned as a large class of conjugated polymer molecules, and more specifically MPS-PPV [2-poly 2-methoxy-5-proplyloxy sulfonate phenylene vineylene), that when used can lead to a greater than "million-fold amplification" of the sensitivity to fluorescence quenching. The Chen article, p. 12290, also states that the results of FIG. 6 provide "demonstration of an attractive and versatile biosensor based on fluoresence recovery from conjugated polymer." [emphasis added] Therefore, the Chen article sets forth specific molecules, namely PPV, that would be capable of functioning as recited in the claim and specification and/or that would be know to one of ordinary skill in the art at the time of the filing of the present invention.

In addition, to further accentuate the plurality of molecules used in the present Application, Independent Claim 1 - Part A has been amended to include the limitations of presently canceled Claim 3, which recites "conjugated polymer molecules." In addition, Dependent Claim 2 has been amended to include "conjugated polymer." Upon this amendment and previous discussion, Claim 1 - Part A is now in condition for allowance.

B. a sample fluid contained within said core region, said sample fluid having a plurality of microorganisms dispersed therein;

With regard to Claim 1 - Part B, the Examiner states that the originally filed specification of the present Application indicates that the claim language "microorganism" encompasses a wide range of potential analytes including biological and chemical agents, such as TNT. The Examiner states that "microorganism" would convey to one of ordinary skill in the art that all of the possible "microorganisms" listed in the specification could be used and would function in the presently claimed invention.

The Applicant presently amends Independent Claim 1 - Part B to replace "microrganisms" with "biological material" to more clearly identify the listed biological and chemical material provided in the specification of the present Application. In addition, Claims 2, 10, 14 and 21 have also been amended to replace "microrganisms" with "biological material." Claims 14 and 15 are amended to further clarify biological microorganism from chemicals such as MPS-PPV. As listed in the specification, "biological microorganisms" would include bacteria, antibodies, cells and proteins. Based upon this Amendment to Claim 1 - Part B, the Applicant submits that Claim 1 - Part B is now in condition for allowance.

C. an optical source for generating excitation light directed to said sample fluid; wherein in response to said excitation light, at least one of said plurality of microorganisms is capable of interacting with at least one of said plurality of molecules so as to generate a fluorescent signal; and

With regard to Claim 1 - Part C, the Examiner states that the specification fails to provide guidance as to any specific combination of "microorganism", excitation light and film molecule that would provide the signal generation of fluorescent light required of the instant claims. Also, the Examiner states that Grey (U.S. Patent No. 5,157,261) and Ligler (U.S. Patent No. 5,496,700) are prior art that are drawn to fluorescence detection systems for the detection of "microorganisms" but neither of these references convey to one of ordinary skill in the art that

either of the detected analytes "interact" with a film of molecules "in response to an excitation light". In view of this information, the Examiner states that one skilled in the art would be required to perform undue experimentation to identify any "microorganisms" and/or "polymer molecules" that would be responsive to excitation light and interact to generate or create a fluorescence signal as required of the instant claims.

The Applicant respectfully traverses the rejection of Claim 1 - Part C for the reasons stated below. As previously discussed, the Chen Article would have been known to one skilled in the art at the time of the filing of the Patent Application. The paragraph below summarizes the utility of the system described in the Chen article at p. 12291.

> "The system described above (and the many possible variations of it) are remarkable from a number of different perspectives. The key component is the ionic conjugated polymer, which leads to two critical effects. First is amplification of the quenching sensitivity, which we attribute to the large number (>1000) of monomer units per chain, and the high mobility of the exciton along the chain to find the quenching site Second, once the quenching reagant has been stripped away by the analyte protein, the relatively large sizes of both the MPS-PPV polymer and the protein prevent further association with the quencher, so that the strong fluorescence can be completely recovered"

Based on the excerpt above, the Chen article provides guidance as to any specific combination of "microorganism", excitation light and film molecule that would provide the signal generation of fluorescent light required of the instant claims. Based upon the Chen Article, the Applicant submits that Claim 1 - Part C is now in condition for allowance.

In the Office Action, the Examiner rejected Claims 1-21 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states "it is not clear what is responsive to the recited excitation light." In addition, the Examiner expounds upon this statement in the Response to Arguments section at para. 13, p. 13 of the present Office Action. The Examiner maintains "that it is not conventional or readily apparent to one of ordinary skill in the art that microorganisms response to excitation light by binding to conjugated polymers.

Applicants have not provided any evidence that this type of reaction is well known or convention to one having ordinary skill in the art."

The Applicant respectfully traverses the rejection of Claims 1-21 under 35 U.S.C. 112, second paragraph as being indefinite. As requested by the Examiner, the Applicant submits the Chen Article as evidence that microorganisms respond to excitation light by binding to conjugated polymers. The Applicant submits that the Chen article was available almost two years before the filing of the present Application and was well known in the prior art at the time of the filing of the present Application. Based upon the Chen Article, the Applicant submits that Claims 1-21 are not indefinite. Therefore, the Applicant requests that Examiner review the rejection of Claims 1-21 under 35 U.S.C. §112.

In summary, there is no proper basis for the Examiner's §112 rejections. Those rejections should be reconsidered and withdrawn. Independent Claims 1 and 21 are now in condition for allowance as well as all the Dependent Claims 2 and 4-20, which directly or indirectly depend from Claim 1.

35 U.S.C. §103(a) Rejection

Grey in view of Broeng

In the Office Action, the Examiner rejected Claims 1, 3-9 and 11-20 under 35 U.S.C. §103(a) as being unpatentable over Grey et al. (U.S. 5,157,261) in view of Broeng et al. (WO 99/64903). The Examiner states that Grey "discloses a fluorescence detection system which includes a fiber optic which includes a surface coated with a plurality of molecules. A sample fluid having a plurality of microorganisms (TNT) dispersed therein is contacted with the coated molecules. The device includes an optical source for generating excitation light and an optical detector for detecting a resultant fluorescent signal."

In the Office Action, the Examiner admits that Grey differs from the present Claims 1,3-9 and 11-20 by not reciting the use of a photonic band gap (PBG) structure with an internal core region for supporting the coated molecules wherein the sample fluid is contacted or contained within the core region. However, the Examiner cites to Broeng that discloses the use of a

photonic band gap structure with an internal core region. The Examiner states it would have been obvious to one of ordinary skill in the art to employ a photonic band gap structure and expected advantages associated with the photonic band gap structure as discussed by the reference Broeng.

The Applicant respectfully traverses the rejection of Claims 1, 3-9 and 11-20 under 35 U.S.C. §103(a) as being unpatentable over Grey in view of Broeng. Both Grey and the present Application contain a number of differences. Grey does not disclose a fluorescence detection system that can act as an optical filter, so that the signal to noise ratio of the detection system can be enhanced considerably. In contrast, the present Application discloses a fluorescence detection system that can act as an optical filter. The fluorescent signal is characterized by a wavelength that falls within the band gap of the PBG structure. The fluorescent signal is thus transmitted through the core region and onto the detector by resonant reflections from the PBG structure. The excitation light is characterized by a wavelength that falls outside of the band gap of the PBG structure, and within a transmission band of the PBG structure. In this way, the excitation light is prevented from being guided through the core region onto the detector. Accordingly, the PBG structure acts as a filter for the fluorescence detetction system.

In addition, the Examiner states that a plurality of microorganisms in Grey relates to TNT which is a chemical microorganism. Independent Claim 1 has been amended to include "biological materials" which is not mentioned in Grey. Therefore, the Applicants submits that Grey would not considered prior art because Grey does not disclose a fluorescence detection system that can act as an optical filter and Grey does not disclose a system for detecting "biological material."

Even if Grey is considered relevant prior art by the Examiner, Broeng in view of Grey, would not be applicable as prior art to the subject matter of Claims 1, 3-9 and 11-20. The Examiner states that Broeng discloses the use of a photonic band gap structure with an internal core region that is not found in Grey. However, Broeng does not disclose the use of PBG structures in a fluorescence detection system that can act as an optical filter, which is also true of Grey. Since it does not teach the subject matter of the present Claims, Broeng is not relevant prior art to the present Application. Therefore, the Applicant submits that Claims 1, 3-9 and 11-

20 are patentable over Grey et al. (U.S. 5,157,261) in view of Broeng et al. (WO 99/64903). Claims 1, 3-9 and 11-20 are in condition for allowance.

Grey in view of Broeng in further view of Walt or Pinkel

In the Office Action, the Examiner rejected Claim 21 under 35 U.S.C. §103(a) as being unpatentable over Grey et al. (U.S. 5,157,261) in view of Broeng et al. (WO 99/64903) take in further view of either Walt et al. (U.S. 5,250,264) or Pinkel et a. (U.S. 5,690,894). Grey in combination with Broeng is not relevant prior art to the subject matter of the present Claim 21 for reasons similar to Independent Claim 1. Also, Walt and Pinkel do not teach the detection of "biological material" as admitted by the Examiner. In addition, Walt and Pinkel teach neither a film with conjugated polymer molecules, nor a PBG structure. Since neither Walt nor Pinkel teach the subject matter of the present Claim 21, Walt and Pinkel are not relevant prior art to the present Application. Therefore, Claim 21 is patentable over Grey in view of Broeng taken in further view of either Walt or Pinkel. Claim 21 is now in condition for allowance.

Ligler in view of Broeng

In the Office Action, the Examiner rejected Claims 1-5, 7-13 and 16-20 under 35 U.S.C. §103(a) as being unpatentable over Ligler et a. (U.S. 5,496,700) in view of Broeng et al. (WO 99/64903). The Examiner states that Ligler discloses a fluorescence detection system which includes a fiber optic with a coated surface with a plurality of molecules. In contrast to the present Application, Ligler does not disclose a fluorescence detection system that can act as an optical filter, so that the signal to noise ratio of the detection system can be enhanced considerably.

In addition, Ligler does not disclose a film comprised of conjugated polymer molecules. The Examiner states merely that the binding molecules are conjugated onto the surface of the optical structure. Since Ligler does not teach the subject matter of the present Claims 1-5, 7-13 and 16-20, it is not relevant prior art to the present Application.

Even if Ligler is considered relevant prior art by the Examiner, Broeng also would not be applicable as prior art to the subject matter of the Claims 1-5, 7-13, and 16-20, for reasons similar to Independent Claim 1. Therefore, Claims 1-5, 7-13 and 16-20 are patentable over

Ligler et al. (U.S. 5,157,261) in view of Broeng et al. (WO 99/64903). Claims 1-5, 7-13 and 16-20 are now in condition for allowance.

Ligler in view of Broeng in view of Walt or Pinkel

In the Office Action, the Examiner rejected Claim 21 under 35 U.S.C. §103(a) as being unpatentable over Ligler at a. (U.S. 5,496,700) in view of Broeng et al. (WO 99/64903) taken further in view of either Walt et al. (U.S. 5,250,264) or Pinkel et al. (U.S. 5,690,894. Ligler in combination with Broeng are not relevant prior art to the subject matter of the present Claim 21, for reasons similar to Independent Claim 1. Walt and Pinkel are also not relevant prior art to the present Application for reasons stated above with regard to the Independent Claim 21. Therefore, Claim 21 is patentable over Ligler in view of Broeng taken in further view of either Walt or Pinkel. Claim 21 is now in condition for allowance.

Conclusion

Based on the foregoing, the Applicants assert that Claims 1-2, 4-21 are now in condition for allowance. If the examiner believes that a telephone conference with the Applicants' attorney would further the prosecution of the application, he is invited to telephone the undersigned at the number listed below.

Previously, a three-month extension of time and Notice of Appeal was requested on June 1, 2006. Prior to the filing of an appeal brief within the two month time period, the Applicant wishes to withdraw the present Application from appeal and to reopen prosecution of the application. Applicant has attached a request for continued examination under 37 C.F.R. §1.114 to this Response and the authorization is hereby given to charge the proper fee set forth under 37 C.F.R. §1.17(e) to our deposit account no. 50-2678.

If additional fees are required, or otherwise necessary to cover any deficiency in fees already paid, authorization is hereby given to charge our deposit account no. 50-2678.

Respectfully submitted,
Greenberg Traurig, LLP
Customer No. 35893

Date: July 11, 2006

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Highly sensitive biological and chemical sensors based on reversible fluorescence quenching in a conjugated polymer

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The fluorescence of a polyanionic conjugated polymer can be quenched by extremely low concentrations of cationic electron acceptors in aqueous solutions. We report a greater than million-fold amplification of the sensitivity to fluorescence quenching compared with corresponding "molecular excited states." Using a combination of steady-state and ultrafast spectroscopy, we have established that the dramatic quenching results from weak complex formation [polymer$^{(-)}$/quencher$^{(+)}$], followed by ultrafast electron transfer from excitations on the entire polymer chain to the quencher, with a time constant of 650 fs. Because of the weak complex formation, the quenching can be selectively reversed by using a quencher-recognition diad. We have constructed such a diad and demonstrate that the fluorescence is fully recovered on binding between the recognition site and a specific analyte protein. In both solutions and thin films, this reversible fluorescence quenching provides the basis for a new class of highly sensitive biological and chemical sensors.

With the rising awareness of the public vulnerability to chemical and biological terrorism, there is a heightened need for detection techniques that show both high sensitivity and selectivity. Such techniques also would find wide use in medical diagnostics and biomedical research applications. Methods of identifying biological molecules such as the enzyme-linked immunosorbant assay (ELISA) achieve selectivity by using specific antibody/antigen interactions to anchor the antigen to a substrate, with a subsequent colorimetric change or fluorescence signal on addition of secondary reagents; these techniques can be time-consuming and require multistep procedures. Other approaches have used molecular recognition ligands to link to specific receptor sites on a biological species, usually as a means also of fixing the biomolecule to a substrate or membrane (1–6) It has remained a challenge to incorporate the selectivity offered by ligand/receptor interactions into a sensor that can be extremely sensitive, robust, and versatile.

We have recently explored the photophysical properties of a fluorescent, water-soluble polyanionic conjugated polymer [poly (2-methoxy-5-propyloxy sulfonate phenylene vinylene (MPS-PPV)] (Fig. 1*B*), one of a larger class of related molecules [poly phenylene vinylene (PPV)] (Fig. 1*A* and derivatives) that has been the subject of almost explosive recent interest (7–13). Although much attention has focused on the well known potential for use of PPV derivatives as electronic materials [e.g., electrochemical sensors (14–16) light-emitting diodes (17, 18), and integrated circuits (19, 20)], the highly charged backbone of MPS-PPV (with charge density approximating that of polynucleic acids such as DNA and RNA), also makes it a model polymer for understanding the interactions and self-assembly properties of charged biopolymers. In this paper, we report a striking discovery: the use of this fluorescent anionic polymer leads to a greater than million-fold amplification of the sensitivity to fluorescence quenching, relative to that of corresponding small conjugated molecules with similar structure. The amplification is attributed to a combination of delocalization of the electronic excited state (exciton) and ultrafast exciton mobility along the conjugated polymer chain. We have harnessed this amplification to demonstrate a versatile new class of highly sensitive (and selective) biological and chemical sensors.

MPS-PPV is a water-soluble polymer, with molecular weight estimated from light scattering measurements to be $1–5 \times 10^5$ (≈1,000 monomer repeat units). The absorption and fluorescence spectra of MPS-PPV in dilute aqueous solution are similar to those of trans-stilbene and its derivatives, but shifted to longer wavelength because of the extended conjugation in the polymer. It is well established that excited states of trans-stilbene and related molecules are readily quenched by electron-deficient aromatic compounds in both dynamic and static processes (21–24).‖ For example, the fluorescence of trans-stilbene derivatives can be quenched by *N*, *N*′-dimethyl-4,4′-bipyridinium (MV^{2+}) (methyl viologen) (Fig. 1*F*) by formation of relatively weak ground-state "donor–acceptor" complexes (21–23). The quenching follows a conventional "Stern-Volmer" relationship:

$$\phi^o/\phi = 1 + K_{SV}[MV^{2+}] \quad [1]$$

where ϕ^o and ϕ are the quantum efficiencies (or intensities) of fluorescence in the absence and presence of MV^{2+}, respectively, and $[MV^{2+}]$ is the MV^{2+} concentration. The constant K_{sv} thus provides a direct measure of the quenching sensitivity. Although the quenching of trans-stilbene by MV^{2+} in homogeneous solution can only be observed at relatively high concentrations of MV^{2+} ($K_{SV} = 15$) (Fig. 2*A*), it is much more easily detectable when trans-stilbene or its amphiphilic derivatives are incorporated into anionic assemblies such as micelles or bilayer vesicles (21–24). The amplification in quenching sensitivity from solution to anionic detergent (sodium lauryl sulfate) micelles ($K_{SV} = 2 \times 10^3$) (Fig. 2*A*) can be readily attributed to a "concentration enhancement" effect in which the stilbene and viologen are assembled by a combination of coulombic and entropic interactions in a microphase such that their "local" concentrations are greatly enhanced (21–23). Given the net negative charge on MPS-PPV, we anticipated that it might readily bind MV^{2+} in aqueous solution and lead to significant fluorescence quenching

Abbreviations: MPS-PPV, poly (2-methoxy-5-propyloxy sulfonate phenylene vinylene; MV^{2+}, (methyl viologen) *N*, *N*′-dimethyl-4,4′-bipyridinium; PPV, poly phenylene vinylene; B-MV, biotin-methyl viologen; MV^+, (mono methyl viologen) *N*-methyl,4,4′-pyridylpyridinium; TA, transient absorption; SE, stimulated emission; PA, photoinduced absorption.

See commentary on page 12219.

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‖"Dynamic" refers to interactions that take place because of diffusion of the fluorophore and quencher species within the radiative lifetime whereas "static" refers to a bound fluorophore-quencher complex. In stilbene-based chromophores and polymers, the short (nanosecond) radiative lifetime leads to a vanishing contribution from dynamic quenching except at extremely high concentrations. For the purposes of our study, these can be neglected.

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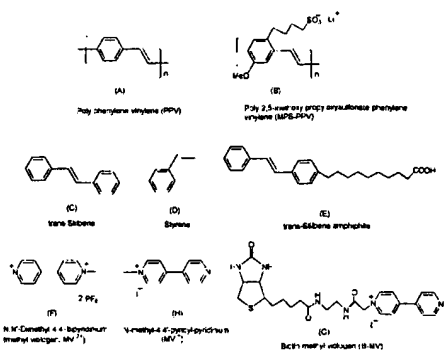


Fig. 1. Chemical structures for the molecules in this study.

at moderate viologen concentrations (Fig. 2*A*). Remarkably, we find that, in dilute solutions of MPS-PPV (10^{-5} M in monomer repeat units), addition of very low concentrations of MV^{2+} leads to noticeable changes in the MPS-PPV absorption spectrum and to a dramatic quenching of its fluorescence (Figs. 3 and 4). The corresponding quenching constant (K_{SV}) is 1.7×10^7, or nearly four orders of magnitude greater than that for stilbene in micelles and six orders of magnitude greater than that for dilute stilbene solutions. As shown by the Stern-Volmer plot in Fig. 4, quenching is nearly (95%) quantitative at 10^{-7} M viologen and is readily detectable at concentrations of 10^{-9} M. Under these conditions, one molecule of MV^{2+} is effectively quenching at a level of ≈1,000 repeat units, or approximately one MV^{2+} molecule per polymer chain! Other quenchers of the "molecular" excited state of trans-stilbene (21–23) are also effective at quenching the fluorescence of MPS-PPV.

The remarkably low levels of viologen and other reagents that are effective in quenching the fluorescence for MPS-PPV may be attributed to several phenomena not generally encountered for molecular excited states or even excitonic states of aggregates. To gain an understanding of the mechanism for this dramatic quenching, the system was studied by using femtosecond transient absorption (TA). The experimental setup used for the TA measurements has been described in detail elsewhere (25). The samples were photoexcited at 3.1 eV, within the π-π^* absorption band of the MPS-PPV polymer. Cross-correlation measurements between the pump and the probe using two-photon absorption in a sapphire plate showed a system resolution time of 150 fs over the entire spectral range studied (25). As a measure of transmission changes, we use the differential transmission (DT), defined as $DT = (T - T_0)/T_0 = DT/T_0$, where T_0 and T are the transmission of the probe beam in the presence and absence of the pump, respectively. In our data, we plot the pump-induced absorption change ($\Delta\alpha$), which is related to DT by the expression $\Delta\alpha = -1/d \ln(1 + DT)$, where d is the sample thickness.

It is well known that the relatively large energy difference between absorption and emission leads to efficient population inversion and lasing in PPV derivatives (26–28); the corresponding stimulated emission (SE) leads to a positive DT signal, and this signal provides a dynamic measure of the exciton population

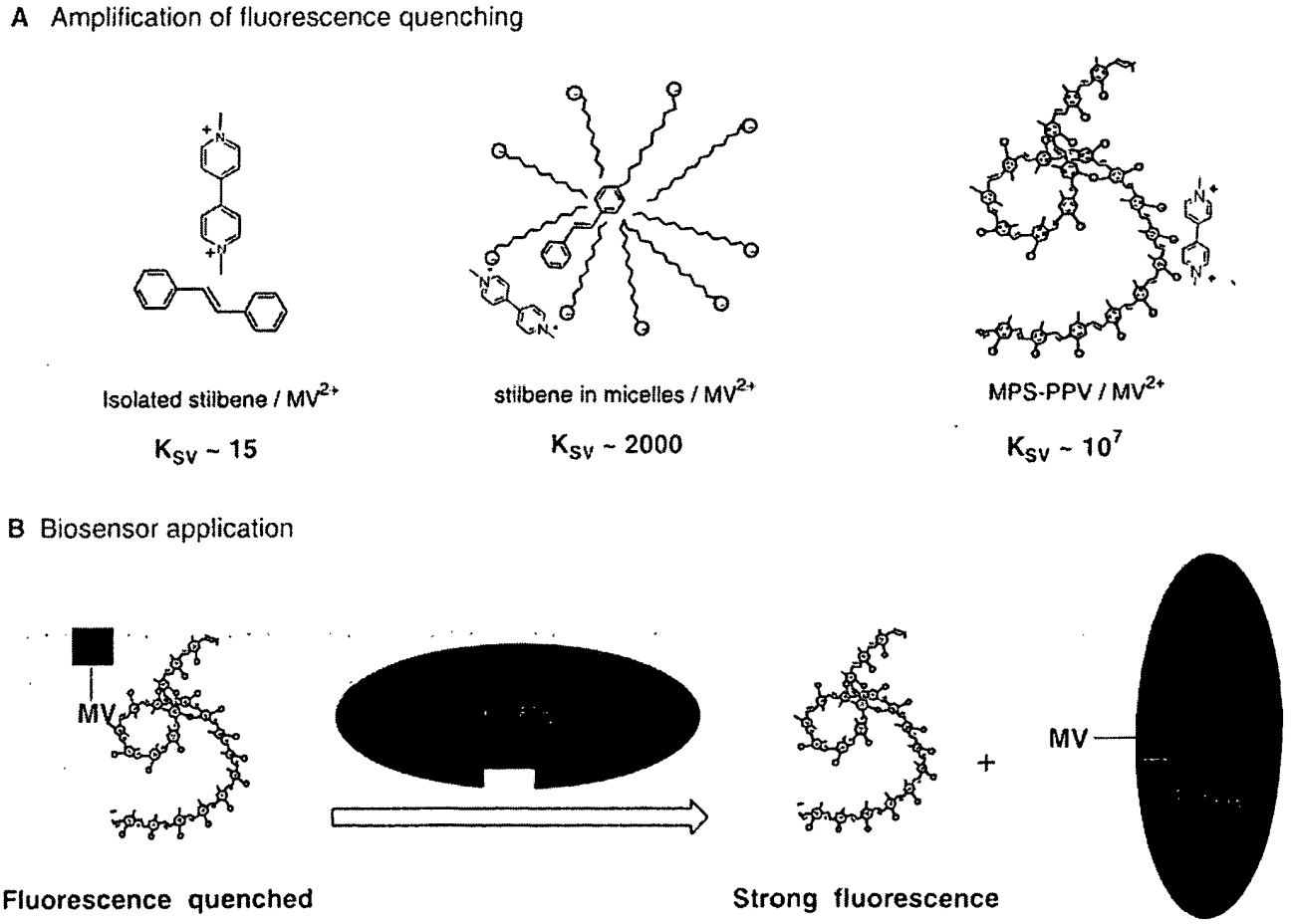


Fig. 2. (*A*) Amplification of fluorescence quenching sensitivity. (*B*) Biosensor application.

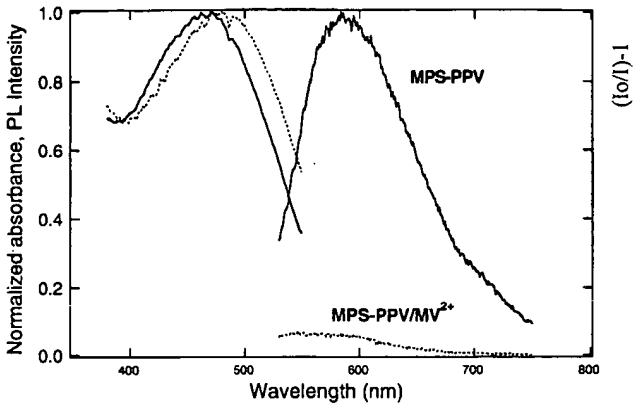


Fig. 3. Absorption and fluorescence spectra (excited at 500 nm) of MPS-PPV (1.7×10^{-5} M in monomer repeat units) in water in the presence (dotted line) and absence (solid line) of MV^{2+} (1×10^{-7} M).

(29–30). It is also known that aggregation of polymer chains in solution and films leads to quenching of excitons by formation of nonemissive interchain excited states (excimers or interchain excitons) (31). The consequences of these processes on the TA dynamics in neat MPS-PPV solutions (1.5×10^{-3} M) are illustrated in Fig. 5*A*. The inset to Fig. 5*A* shows the TA spectrum from 450–750 nm, showing both SE (positive $\Delta T/T_0$) and photoinduced absorption (PA) (negative $\Delta T/T_0$) bands, attributable to the photoinduced transition of the exciton back to the ground-state, or to a higher excited-state, respectively. In the first 2 ps, the spectrum decays, with an apparent blue shift attributable to the formation of secondary interchain excited-states, with a competing PA (see the difference spectrum in the inset to Fig. 5*A*). The temporal evolution of excitons to interchain excited-states (excimers) can be directly monitored by comparing the dynamics near the peak of the SE (500 nm) and near the zero crossing of the TA spectrum (600 nm, where the exciton cross-section is nearly zero, but the interchain state has a finite PA) as shown in Fig. 5*A*; the initial decay of the SE (exciton) has a time constant of 1.5 ps, and there is a complementary growth of the excimer population with the same time constant. Hence, aggregation of MPS-PPV at these relatively

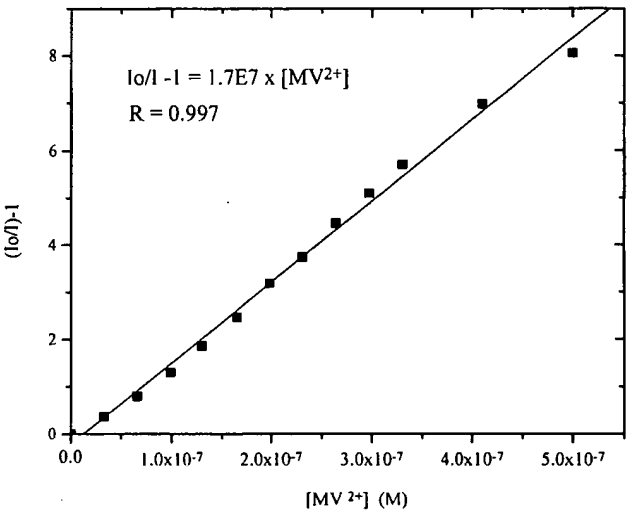


Fig. 4. Stern-Volmer plot for quenching of the fluorescence of 1.2×10^{-5} M (repeat units) MPS-PPV by MV^{2+} in aqueous solution.

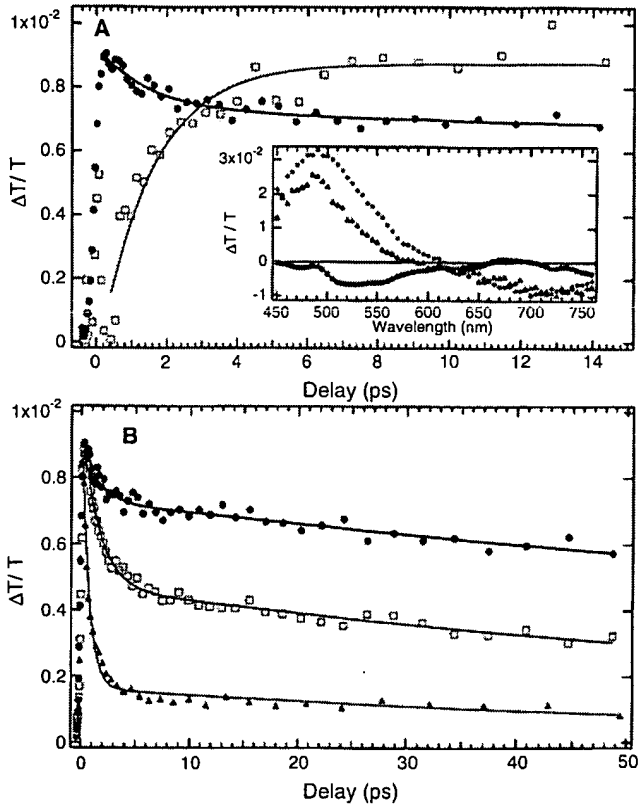


Fig. 5. (*A Inset*) TA spectra in 1.5×10^{-3} M MPS-PPV solution at zero time delay (circles) and 2-ps delay (triangles), showing SE peak (500 nm) and PA peak (720 nm), together with difference spectra showing secondary PA peak caused by excimer formation in the aggregated polymer. (*A*) SE decay (circles) and excimer PA growth (squares) showing complementary dynamics and 1.5-ps decay/growth time. (*B*)SE decay in 5×10^{-4} M MPS-PPV solution before (circles) and after addition of 1×10^{-5} M MV^{2+}, with equilibration of solution (squares) and after agitation (triangles).

high concentrations provides a direct quenching mechanism in neat MPS-PPV solutions. Fig. 5*B* compares the decay of the SE (500 nm probe) in a 5×10^{-4} M MPS-PPV solution, with that for the same solution with addition of 10^{-5}M MV^{2+}. When the MPS-PPV/MV solution is allowed to equilibrate for several minutes, the dynamics show an increase of the 1.5-ps decay component, with no change in the initial 1.5-ps lifetime. This indicates that the dicationic MV^{2+} is promoting additional aggregation of the relatively concentrated MPS-PPV solutions. Interestingly, agitation of the solution (either by gentle shaking or sonication) leads to a dramatic increase in both the magnitude and the rate of SE quenching, with a time constant of 650 fs. The evolution between these two types of dynamics is fully reversible. This dramatic change in the ultrafast exciton decay points to two competing quenching mechanisms: aggregation quenching caused by formation of interchain states and electron-transfer quenching caused by the MPS-PPV/MV^{2+} complex. The addition of divalent cations to anionic polyelectrolytes is known to lead to aggregation (32), and, hence, the MV^{2+} intrinsically plays a dual role. The fact that other non-electron-deficient divalent cations such as Ca^{2+} and Mg^{2+} (which do not quench stilbene) also quench the MPS-PPV emission, but not as efficiently as MV^{2+} (K_{SV} for Ca^{2+} is 10^4), supports this picture of competition between aggregation and electron-transfer quenching. It is also significant that addition of monovalent cations (K^+ and Na^+) (which do not promote aggregation) had a negligible quenching effect. It is important to note that, at MPS-PPV/MV concentrations used in Fig. 3 and elsewhere in this study, no changes in

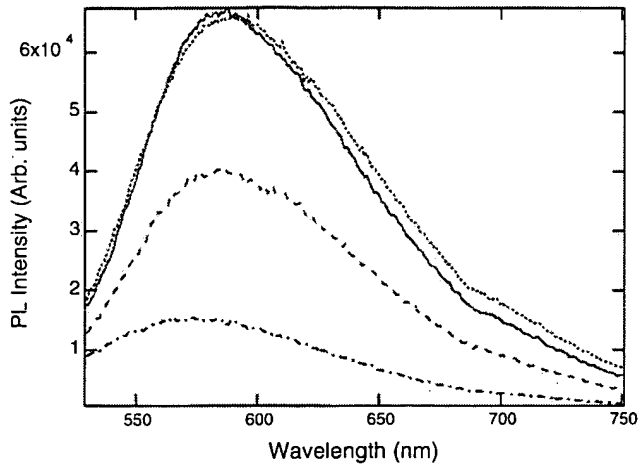
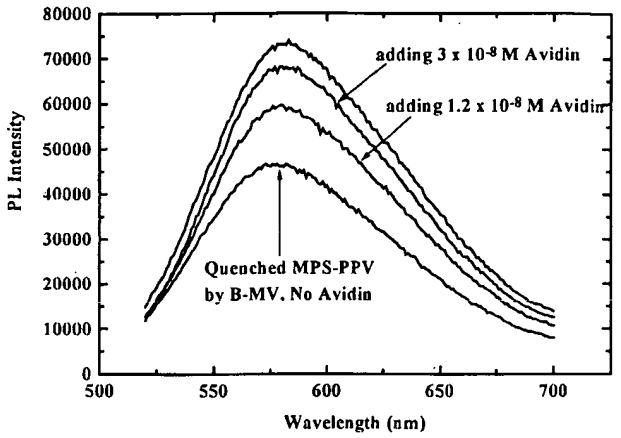


Fig. 6. Fluorescence spectra from aqueous solution of MPS-PPV (1.7×10^{-5} M in monomer repeat units) excited at 500 nm in water alone (solid line), after addition of 2×10^{-6} M B-MV (dash-dot line), after addition of 1×10^{-7} M avidin (dash line), and after addition of 2×10^{-7} M avidin (dot line).

the quantitative quenching were observed with time, and agitation was not necessary to achieve efficient quenching. Hence, the aggregation quenching likely plays a minor role for MV^{2+} at low concentration.

Because the ground state binding of trans-stilbene and even negatively charged assemblies containing trans-stilbene derivatives by MV^{2+} is relatively weak (21–23), it was interesting to determine whether the highly effective fluorescence quenching observed in the presence of viologen and other cationic reagents could be reversed. An attractive possibility involves the synthesis and use of a molecule in which a viologen-type quencher and a second recognition unit were combined, separated by a relatively short "tether." Accordingly, we prepared biotin-methyl viologen (B-MV) (Fig. 1*G*), which combines a viologen unit linked to a biotin molecule by a short but flexible tether. Biotin is an excellent receptor for proteins such as avidin and streptavidin, but it was not expected to react with MPS-PPV (33–35). The complex between biotin and avidin is extremely strong ($K \approx 10^{15}$ M) (36), and, consequently, the binding is expected to be rapid and effectively irreversible. The avidin-biotin complexation has been very well studied (and used in several biomedical diagnostic assays) (37); avidin contains four biotin binding sites, and the protein has a molecular weight of ≈64,000 (38). Consequently, it was anticipated that, in the absence of receptor protein (avidin), the small biotin group in B-MV would not hinder association of the viologen portion of B-MV with MPS-PPV and that its addition to solutions of MPS-PPV would result in strong fluorescence quenching. Because the protein is a much larger molecule than either biotin or MPS-PPV, and because protein-biotin complexation should be much stronger than that for the polymer-viologen combination (33–35, 38), we anticipated that addition of protein to these "quenched" solutions might reverse the quenching (Fig. 2*B*).

Indeed, as shown in Fig. 6, addition of B-MV to solutions of MPS-PPV results in quenching of its fluorescence. B-MV is somewhat less effective as a quencher than MV^{2+}, which is reasonable attributable to its lower positive charge; its quenching is quite comparable to that of the "mono" viologen cation (mono methyl viologen) *N*-methyl,4,4′-pyridylpyridinium (MV^{+}) (Fig. 1*H*). Addition of very small amounts of avidin reverses this quenching, as anticipated in Fig. 2*B*. As shown in Fig. 6, the amount of avidin necessary to produce significant fluorescence recovery is remarkably low. Partial quenching and reversal may be demonstrated by using even lower concentrations of quencher



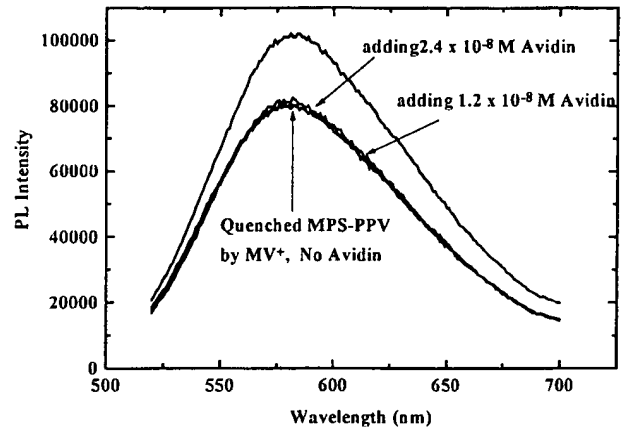


Fig. 7. Partial quenching of fluorescence of MPS-PPM (10^{-5} M in "repeat units") by B-MV (*Upper*) and MV^{+} (*Lower*) (quencher concentration 3.2×10^{-7} M in each case). In the upper plot, the fluorescence quenching is reversed by addition of 1.2×10^{-8} M and 3×10^{-8} M avidin whereas addition of similar amounts to the MV^{+}-quenched sample (*Lower*) shows no increase.

and protein. Fig. 7 shows that addition of either MV^{+} or B-MV at $2–3 \times 10^{-7}$ M produces significant quenching of the fluorescence of MPS-PPV; the fluorescence quenching is reversed in the case in which avidin (1.2×10^{-8} M) is added to the B-MV-quenched sample. However, no change occurs when the same amount of avidin is added to the MV^{+}-quenched sample, verifying that the reversal of fluorescence quenching is caused by the avidin-biotin complex formation. The levels of avidin "sensed" by the recovery of polymer fluorescence are in reasonable accord with an expectation of nearly complete complexation of avidin with a four-fold equivalent of B-MV. Based on these experiments, it is evident that nanomolar amounts of avidin (or lower) may be sensed by this process. Addition of dilute solutions of avidin (2×10^{-7} M) does not produce any detectable change in the fluorescence of "unquenched" MPS-PPV. Furthermore, addition of aqueous solutions of cholera-toxin protein (which lacks a biotin binding site) to B-MV-quenched MPS-PPV produces no increase in the MPS-PPV fluorescence. These results provide strong evidence that the recovery of fluorescence shown in Figs. 6 and 7 occurs as a consequence of the specific biotin-avidin interaction. Taken together, these results for this nonoptimized case provide demonstration of an attractive and versatile biosensor based on fluorescence recovery from the conjugated polymer.

The system described above (and the many possible variations of it) are remarkable from a number of different perspectives. The key component is the ionic conjugated polymer, which leads to two critical effects. First is amplification of the quenching sensitivity, which we attribute to the large number (>1,000) of monomer units per chain, and the high mobility of the exciton along the chain to find the quenching site. Second, once the quenching reagent has been stripped away by the analyte protein, the relatively large sizes of both the MPS-PPV polymer and the protein prevent further association with the quencher, so that the strong fluorescence can be completely recovered. The strategy of using a relatively small amount of a quencher-recognition molecule such as B-MV, and MPS-PPV or a similar conjugated polymer as the optical transduction element, results in a sensing device that may be effectively in the "off" position (near zero fluorescence background) in the absence of the reagent to be sensed. The very short lifetime of the excited states of quenched polymer (<1 ps) should result in relatively little "photobleaching" in the absence of the molecule to be sensed and thus to a potentially robust sensor. The sensitivity and generality of the fluorescence quenching of MPS-PPV (and related polymers) by a wide family of acceptors, and its ready reversal by what is probably best described as a steric effect when the second recognition element binds to the protein, suggests that the approach outlined here may be applicable to a wide variety of specific sensing applications for proteins and other biological macromolecules.

The potential sensing applications of MPS-PPV and related polymers (refs. 39–43, ref. 44 and references therein, and refs. 45–49) are not confined to ionic species or solutions. Neutral, electron-deficient aromatics such as 9,10-dicyanoanthracene and nitroaromatics quench in aqueous solutions at higher concentrations than for MV^{2+} but still at levels where no "dynamic" quenching could occur given the short (≈1 ns) lifetime of the fluorescent state of MPS-PPV. Even more remarkable, quenching is observed for these compounds in solid films of MPS-PPV. As a demonstration, single monolayer films of MPS-PPV were prepared on glass substrates by using polyelectrolyte self-assembly, as described in more detail elsewhere (50). These films show similar fluorescence and absorption to the solutions of MPS-PPV (Fig. 8); interestingly, exposure of these films to the vapor of nitroaromatics such as nitrobenzene or dinitrotoluene leads to substantial quenching of the fluorescence from the films. Fig. 8 shows the rapid quenching that can be observed from dinitrotoluene vapor at room temperature. From the vapor pressure of dinitrotoluene, it can be determined that the film senses (by fluorescence quenching) the nitroaromatic at a level of $<8 \times 10^{-9}$ M. Because the films of MPS-PPV may be readily overcoated with other films of varying thickness and composition, it should be possible to develop a variety of vapor-based "chemical" sensors of high sensitivity and selectivity.

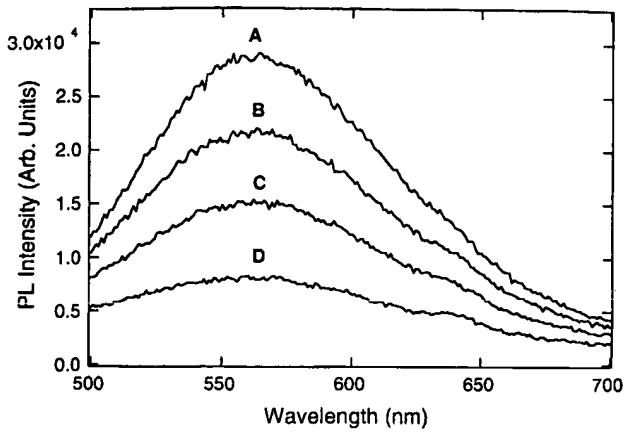


Fig. 8. Fluorescence of a solid film of MPS-PPV excited at 400 nm on exposure of dinitrotoluene vapor as a function of time before exposure (A) and at 10 s (B), 30 s (C), and 60 s (D).

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